

ELECTRON MICROSCOPIC OBSERVATIONS ON THE ADRENAL GLAND OF THE GREEN TOAD (*BUFO VIRIDIS* LAUR.)

A. ÁBRAHÁM

Department of Zoology, Attila József University, Szeged

(Received on December 2nd 1971)

The adrenal gland of the green toad is a yellowish, narrow, band-like body of golden shine, running about in the middle of the ventral side of kidney, from the anterior pole until the posterior one, approaching the latter one strongly without reaching it, anyway. There are sporadically thickenings of smaller or larger extent in it and it occurs, too, that a wide, orally lying initial piece is fastened to the broader substance of caudal position with a relatively long and thin part.

Materials and Methods

We have used fixed and stained sections for orientation. For producing them freshly removed adrenal gland was fixed in CARNOY's and BUIŃ's fluid and stained with hematoxylin, eosin, and phloxin. For the electron microscopic investigations small pieces were excised from the adrenal glands of the decapitated animals, fixed — after being prefixed in glutaraldehyde — in osmiumtetroxide buffered with collidin, dehydrated in alcohol and embedded in araldit. The sections were made with ultra-microtome LKB, studied with electron microscopes TESLA D 242 and JEM 6. The investigations have been carried out partly in the Biological Research Institute of the Hungarian Academy of Sciences Tihany, partly in the Central Medical Research Institute in Budapest, partly in the electron microscopic laboratory of the Attila József University in Szeged.

Observations

The adrenal gland is fully grown together with the kidney. It is closely connected with the initial branches of *venae renales revententes* that take their origin from its substance. Histologically it consists of a system of massive trabeculae that, anastomosing with each other, form a wide-meshed network. The diameter of trabeculae is variable. Here and there they become thick. elsewhere they become thin in a longer part, too.

The cells forming the trabeculae belong to two major groups. One of them appears after being stained hematein-eosin a long-shaped white island containing several round nuclei; the other is a roundish, sometimes angular or elongated, dense node, the polyhedric, resp. elliptical cells of which contain small roundish granules stained strongly with hematein and with eosin, as well. The components of the whitish islands are the lipid cells, those of the dense nodes the chromaffin cells.

Lipid cells

The lipid cells are long-shaped, narrow, their cell membrane being imperceptible under a light microscope, their cytoplasm is colourless and homogeneous, in some instances somewhat foamy. In living state and after being fixed, as well, they contain plenty of fat which, however entirely disappears from the cytoplasm as a consequence of a treatment with alcohol and xylol. The lipid cells are, as to their structure and function, homologous and analogous with the cells forming the cortical substance of the adrenal gland of *Mammalia*.

The lipid cells are under an electron microscope roundish, polymorphous resp. elongated cells. The cell membrane is sporadically sharp, elsewhere blurred and sometimes it seems even so, as if the adjacent cells had melted into each other. The cytoplasm is of foamy stucture because a great deal of large

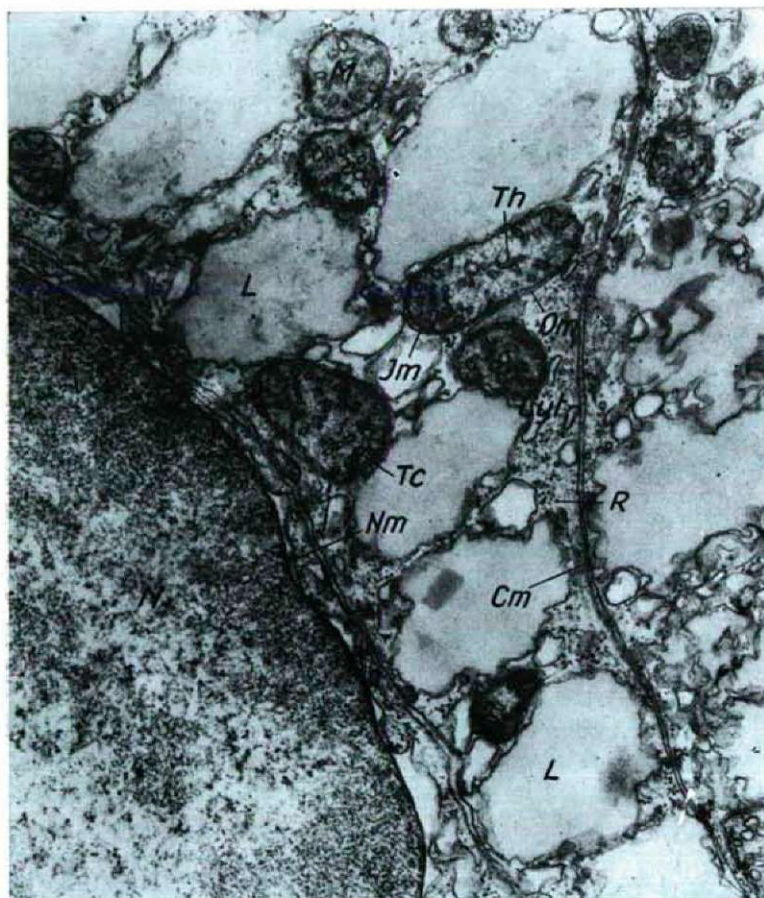


Fig. 1. *Bufo viridis* LAUR. Lipid cells from the adrenal gland. Cyt./cytoplasm, M./mitochondrion, Om./outside membrane, Im./inside membrane, Th./longitudinal section of the tubule, Tc./tubule cross-section, R./ribosome, N./nucleus, Nm./nuclear membrane, L./lipid drop. Magnified: x 25.000

lipid bodies with rugous walls had piled up in it containing lipid substances resolved during the treatment. The substance of lipid bodies is homogenous, an electronic light substance, scattered in some cases with darker, amorphous corpuscles.

The spaces lying between the lipid bodies are filled up with mitochondria. The latter ones are long-shaped roundish bodies, limited by a double wall of sharp contour. In their stroma the tubuli of various length and of approximately uniform diameter manifest themselves in the form of a loose meshwork or in a

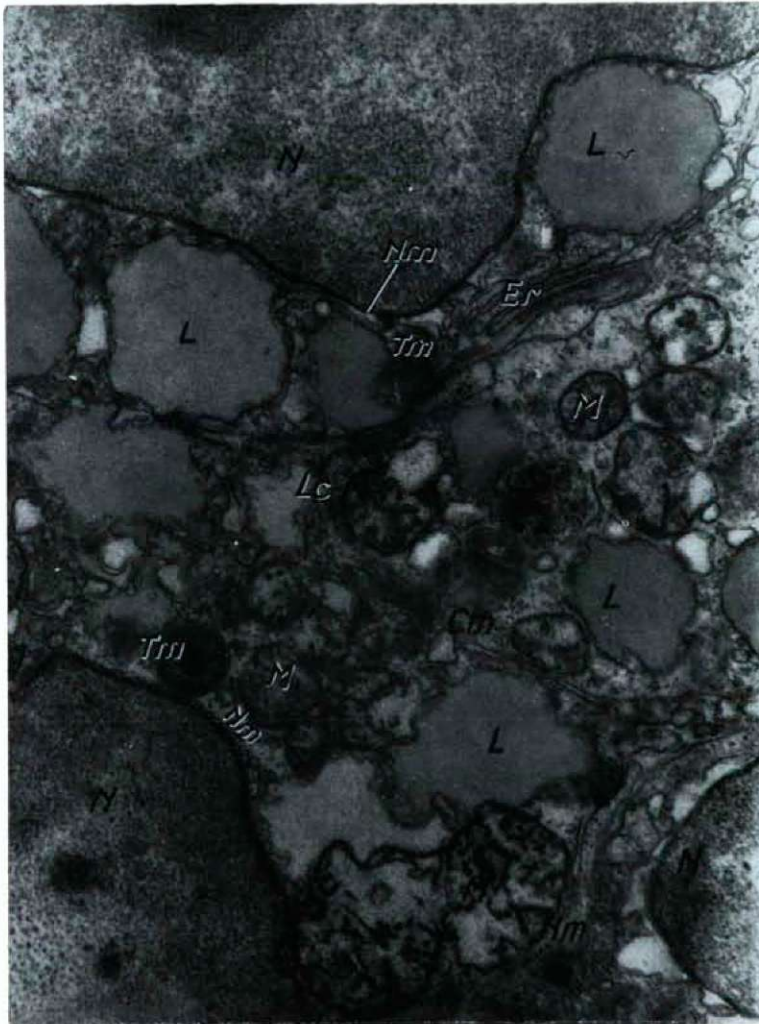


Fig. 2. *Bufo viridis* LAUR. Mitochondria in state of transformation in the cytoplasm of the lipid cell. Lc./lipid cell, M./mitochondrion, Tm./mitochondrion in state of transformation, L./lipid drop, Er./endoplasmic reticulum, N./nucleus, Nm./nuclear membrane, Cm./cell membrane. Magnified: x 25.000

parallel site, forming the main part of the substance. The tubuli are either longer formations with smooth walls of uniform thickness or they are composed of varix-like thickened pieces, connected together with thinner intermediary sections. Their cavity is proportionately spacious. They show the most various forms and sites even within the same body. In the lipid cells the mitochondria are obviously large and are present in a very great mass (Fig. 1).

There are particularly interesting and striking the pictures that are supporting the supposition that the tubules of mitochondria melt together in some period of the development of lipid cells that may be in connection with one of the degrees of ontogenesis, with seasonal situation or with the sexual life. They merge and change gradually into a homogeneous amorphous body in

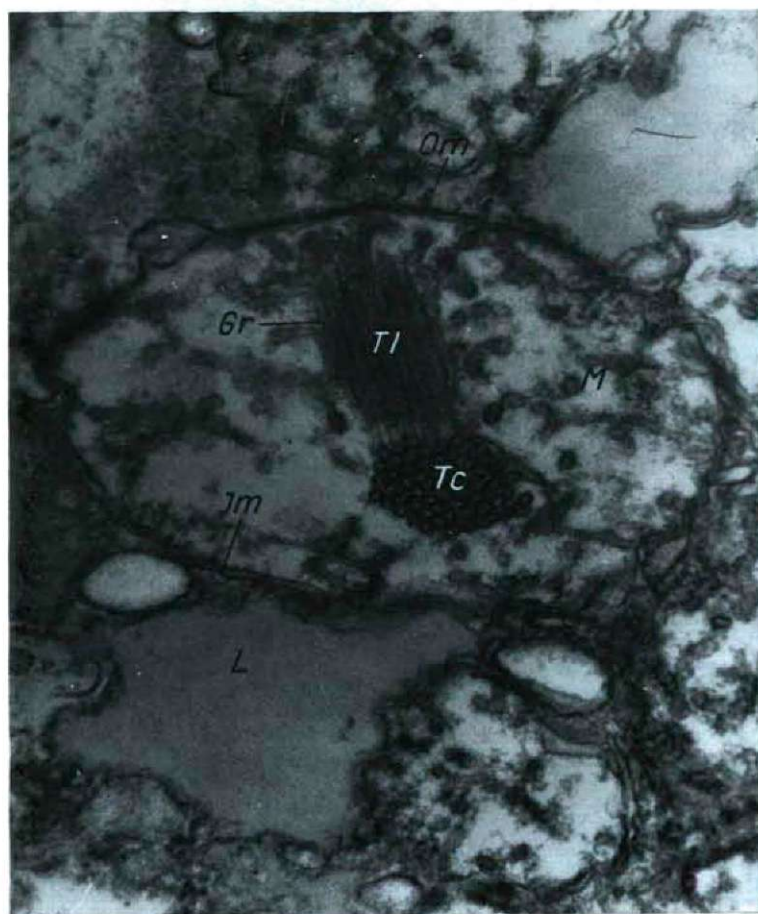


Fig. 3. *Bufo viridis* LAUR. Mitochondrion from the lipid cell. M./mitochondrion, Om/outside membrane, Im./inside membrane, Tl./tubes longitudinally, Tc./tubes in cross-section, Gr./granules, L./lipid drop. Magnified: x 112,000

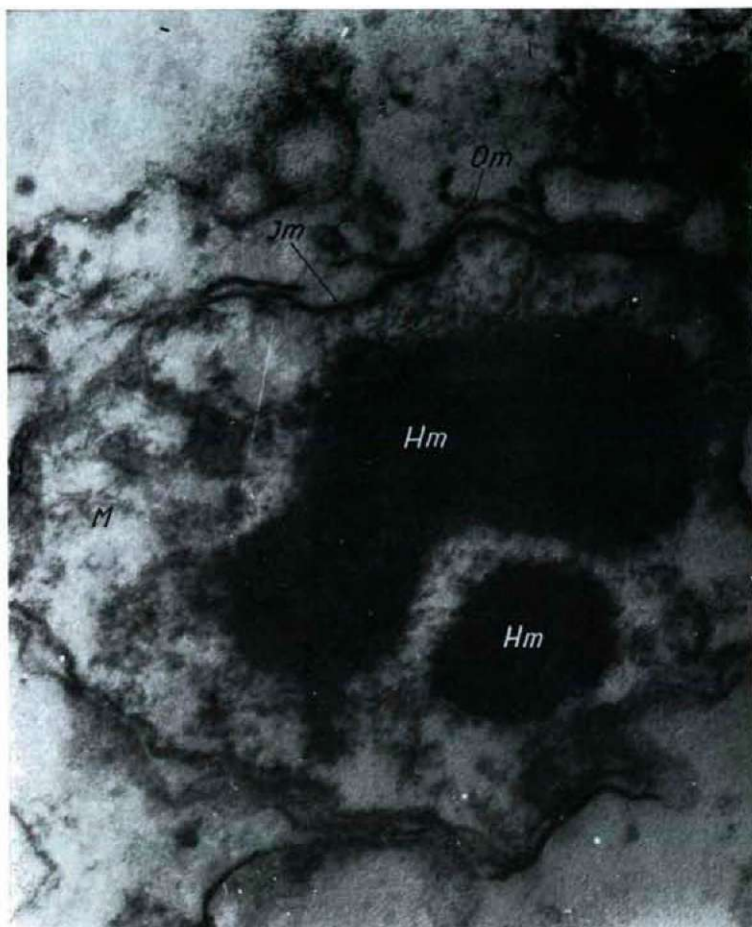


Fig. 4. *Bufo viridis* LAUR. Melted mitochondrial tubules in the lipid cell. M./mitochondrion, Om./Outside membrane, Im./inside membrane, Hm./homogeneous electron dense mass. Magnified: $\times 112,000$

which the site and shape of the single tubules cannot be distinguished any more from one another (Fig. 2). In respect of the fact that these homogeneous strongly electron dense bodies of various form are brought about by the transformation and melting of tubuli, we may be convinced by the pictures in which the process of transformation can be followed from the tubules arranged in bundles until the amorphous homogeneous body.

The transformation began by the incipient straightening out and ranging of the tubuli of winding course. Later on, they form dense bundles in which the single homogeneous tubules can be recognized well (Fig. 3). And even in the pictures magnified strongly there appear some vesicles, resp. granules arranged in lines along these tubules and in their walls respectively whose

physiological role is still needing an explanation. The tubules have later disappeared and a homogeneous dark body has remained in the place of the bundle that could be unitary or divided into parts, neither the structure nor the physiological role of which is known before us (Fig. 4).

As the material of which the sections were made is of November origin, it is imaginable that the phenomenon is in connection with the degradation of metabolism and oxidative reactions. At any rate, the transformation as a whole is unknown, its further investigation is desirable and it seems to be necessary for recognizing the part played by mitochondria.

We must not leave unsaid, either, that — on the basis of looking at some pictures — we could also suppose that the tubules arranged in bundles split up and detach in the shape of vesicles, resolve and are used in the mitochondrion itself or elsewhere. In that case we have to give up the connection between the arrangement of tubuli and the formation of the amorphous body and the way of the formation of this is to be looked for in another way (Fig. 5).

The GOLGI apparatus takes place immediately under the cell membrane. It is a system of wide extent, consisting of several narrow tubules which are long and form a number of windings. Here and there they converge being strangulated at the end in the form of a ball. The phenomenon reminds us of the part attributed to the GOLGI tubuli producing the neurosecretory granules. It is not impossible that the tubuli of GOLGI complex play some part here as well as in the neurosecretory cells of the nervous system (SCHARRER et al., 1961; BLOCH et al., 1966; ÁBRAHÁM, 1969) in producing the dense core granules.

The cisternae of the endoplasmic reticulum take place round the nucleus. They are essentially longer or shorter tubules of changing lumen that expand in some places and form cavity systems of considerable size at some distance from the nucleus. Peculiar forms of the ducts of the endoplasmic reticulum are those resulted from the ramification of a thick tube. This may be the single mark in which the epithelial cells of the convoluted tubules are approached structurally by the lipid cells. In the former ones the ducts of the endoplasmic reticulum appear in a particular sharpness and form, falling into pieces at the end, forming chains from the single particles, transformed into a flat end piece expanded shovel-like (Fig. 6).

The cell nucleus is a roundish, sometimes polyhedric body of central site. It may sometimes have wide and deep indentations with the cytoplasm penetrated into them. The chromatin substance is granular. The double nuclear membrane is usually obvious. The gap between the two membranes is proportionally wide. It occurs, too, that between the two nuclear membranes smaller or larger vesicle-like formations can be seen, limited by walls (Fig. 1).

Chromaffin cells

The chromaffin cells are stained dark by means of hematein. Their structure and function correspond with those forming the medullary substance of the adrenal glands in *Mammalia*. They are full of granules stained well by hematein and eosin, filling up the whole cell, and sometimes it seems even so,

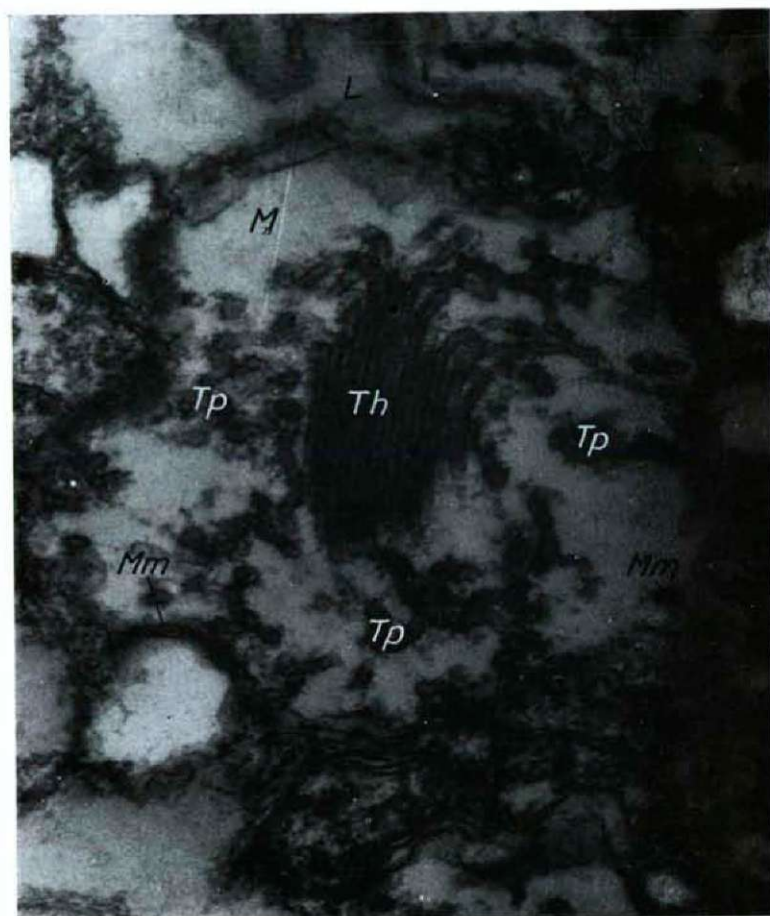


Fig. 5. *Bufo viridis* LAUR. Disintegrating mitochondrial tubules in the lipid cell. M./mitochondrion, Mm./membrane of mitochondrion, Th./mitochondrial tubes in longitudinal section, Tp./disintegrating tubule pieces, L./lipid drops. Magnified: $\times 140.000$

as if the cell were transformed wholly into granules falling to pieces holocrine-like. This seems to be supported by our preparations made of the materials of May and July respectively. The number of granules is much lower in May than in July. The roundish and bright granules encircled at their edge with a thin border and stained strongly by means of eosin manifest themselves mainly after being fixed according to CARNOY.

The chromaffin cells, named sometimes also phaeochromocytes, are roundish, in some cases elongated, at both ends sharp-pointed cells under an electron microscope. The cell membrane is sharp, sometimes strongly wavy. The cytoplasm contains plenty of osmiophilic bodies. These are dense homogeneous formations in pictures less magnified, but at a stronger magnification we see

that they are, if also not all of them, composed of fine roundish granules. The osmiophilic bodies are sometimes surrounded by a wide, homogeneous membrane, growing in some places narrower. These forms remind us of the „dense core” vesicles known from the chromaffin cells in the adrenal gland of the higher *Vertebrates*. Their size is changing but the differences are not great. In the pictures of high power of magnification, the homogeneous forms and those consisting of a multitude of small roundish particles appear side by side.

The cytoplasm between the osmiophilic bodies is homogeneous, in some



Fig. 6. *Bufo viridis* LAUR. Kidney epithelial cell from the wall of the convoluted tubule. Bm./Basal membrane, Cyt./cytoplasm, M./mitochondrion, Om./outside membrane, Im./inside membrane, Cr./crista, Er./endoplasmic reticulum, V./vesicle, R./ribosome. Magnified: x 58.000



Fig. 7. *Bufo viridis* LAUR. Chromaffin cells from the adrenal gland. Cyt./cytoplasm, Og./osmiophilic body, L./lipid drop, M./mitochondrion, N./nucleus, Nl./nucleolus, Nm./nuclear membrane, I./invagination, Sy./synapsis, Sv./synaptic vesicle, Sm./synaptic membrane. Magnified: $\times 24,000$

cases with small granules. Here and there, we can see in it the tubules of the endoplasmic reticulum, taking place generally in the vicinity of nucleus. The lumen of the tubules is changing, there can be seen in them smaller and larger dilatations, and various ramifications are frequent, as well. In the cytoplasm there are not rare some longshaped dense band-like bodies, sharp-pointed at one end and lying in the neighbourhood of the nucleus. The GOLGI complex appears but seldom in the pictures and, if at all, in the form of vesicle groups (Fig. 7).

Acidophilic cells

The workers dealing with the histological investigation of the adrenal gland in frogs distinguish, as a rule, besides the lipid and chromaffin cells that form the main mass of the trabeculae, also a third kind of cells, named owing to their acidophily, acidophilic, resp. eosinophilic or summer cells.

The acidophilic cells are mentioned first by STILLING (1886). He found so that in the adrenal gland of the *Rana esculenta* in summer new cells appear that are missing in spring, autumn and winter. These so-called summer cells are pear-shaped, their cytoplasm is strongly granular, the granules are pronouncedly eosinophilic but well-stained by safranin, hematoxylin and dahlia, as well. Their division is mitotic, they often surround the phaeochromocytes but can easily be distinguished from them. STILLING had seen in these cells a third kind of cells that can not be traced back either to adrenal or to interrenal elements. STILLING had connected the summer cells with Summer and sexual function. On the other hand, BONNAMOUR and POLICARD (1903) had found so that the summer cells are to be found in the winter frogs, as well. GRYNFELLT (1903, 1904) had also found these cells during the whole of the year but only at the „*Ranae*”. He had not found them at other *Anura* (*Hyla*, *Bufo*, *Bombinator*) and *Urodela*.

PATZELT and KUBIK (1912) name the summer cells acidophilic cells and consider them a modification of the epithelial interrenal cells. JONA (1914) had found the summer cells in the whole of summer but only at the *Rana esculenta* and *Rana temporaria* and never at other *Anura* species. He considers the summer cells as leukocytes that got from the lymphoid tissue into the adrenal gland. CARL (1916) had obtained a more intensive granule stain with safranin than with eosin and therefore he does not approve of the denomination „acidophilic”.

RADU (1931) investigating the exemplares of *Rana temporaria* and *Rana esculenta* from January until September, had found the summer cells in the winter exemplars, as well. According to him, the summer cells are immigrated leukocytes that underwent a transformation in the adrenal gland. This opinion of RADU was based on that he had found typical summer cells in the blood vessels and in the intersticium as well. According to him the summer cells, being equivalent with the basophilic leucocytes. The possibility that the summer cells were connected with the sexual life, mentioned before by CIACCIO (1903), is thoroughly rejected by RADU. KUCNEROWICZ (1935) does not regard the summer cells as leukocytes but as particular forms of the adrenal cell type that are showing cyclical changes in secretion and storage in the different seasons.

MARIO H. BURGOS (1957) investigating the histological structure of the adrenal gland in the *Rana pipiens* with electron microscope, as well, took the part of three cell types, resp. he regards the acidophilic cells as a particular cell type of the adrenal substance. According to his description the acidophilic cells found in the adrenal gland of *Rana pipiens* are roundish cell forms of minor size. Their nucleus is of central site and ovoid, in their plasma there are large and strongly eosinophilic granules.

We have seen so both in the May and in the July material of ours that the chromaffin cells are equally densely granular, and in their external appearance there are no major differences to be observed. On the other hand, in our November material under electron microscope we have found, although in lower number, some cell forms that agree in some marks with those of the forms described by MARIO H. BURGOS with an electron microscope from the adrenal gland of *Rana pipiens* (Fig. 8). But there are essential differences

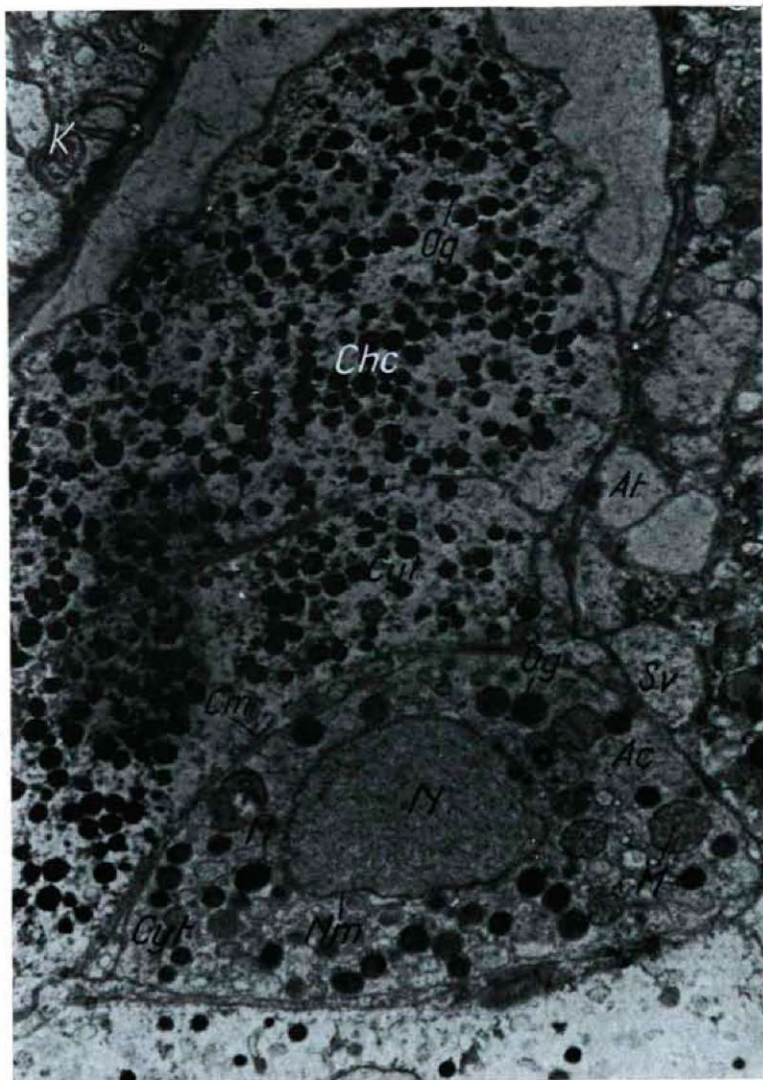


Fig. 8. *Bufo viridis* LAUR. Acidophilic cell and chromaffin cells from the adrenal gland. Ac./acidophilic cell, Chc./chromaffin cell, Cyt./cytoplasm, M./mitochondrion, Og./osmiophilic granule, N./nucleus, Nm./nuclear membrane, At./axon, Sv./synaptic vesicle, K./kidney. Magnified: $\times 24,000$

between the two cell forms that can be summarized as follows: 1. In BURGOS's cells, belonging to the third type and named acidophilic ones, the osmiophilic bodies are about four times as large as those in the chromaffin cells. In the cells found by us, the osmiophilic bodies are not more than two times as large as those in the chromaffin cells. 2. In BURGOS's cells, in the juxtannuclear zone there are no osmiophilic bodies and some bundles consisting of fine filaments are frequently to be seen. In our material, there are osmiophilic bodies in the juxtannuclear zone, as well, but there are no filamental bundles. 3. In BURGOS's cells the mitochondria are extremely small and their number is low. In our cells, the mitochondria are of the same size, and even larger than those in the chromaffin cells and their number is not lower, either, but quite the contrary, it is much higher than in the chromaffin cells.

As mentioned above, between the cell form qualified by MARIO H. BURGOS as a third type and that found by us there are unquestionably great differences but also the animals and the environment were different. One thing seems, anyway, to be sure and that is that both at *Rana pipiens* and at *Bufo viridis* there may be distinguished two kinds of cells in the adrenal substance that differ from each other in the general outline. One of them is the typical chromaffin cell, the other the adrenal cell that is smaller in size and contains larger osmiophilic bodies and more mitochondria. In our opinion, in one of them adrenaline and in the other one noradrenaline is produced.

As it is supported by the physiological investigations (CUPLAND, 1966) that in the adrenal gland of frogs there is produced noradrenaline in disproportionately larger amount than adrenaline, we think that noradrenaline is produced by the chromaffin cells and adrenaline is produced by the acidophilic cells, resp. by those of third type.

It is natural that the electron microscopic investigations carried out so far are not enough to ascertain the definite recognition marks of general character concerning the structure of cells belonging to the third type, they suffice, however, for establishing the presence of such cells and for qualifying them as parts of the adrenal substance. For obtaining an unequivocal and generally valid answer to that question, the investigations must be continued on a comparative basis, comprising all the classes of Vertebrata and all the seasons.

Nerve cells

We have not found any nerve cells either in the preparations stained, or in the electron microscopic pictures. We don't want to say, of course, that they are missing, as the authors dealing with the histological structure of the adrenal gland in frogs report on these, too, as the components of adrenal gland. And another cause for not taking a negative view concerning the occurrence of nerve cells is that in our silver impregnated preparations made of the adrenal gland of *Rana ridibunda* also we have observed some nerve cells and small ganglions that, concluding from their appearance, belong to the sympathetic system.

Nerve fibres

MARIO H. BURGOS (1959) found myelinated fibres among the chromaffin cells in the adrenal gland of *Rana pipiens*. We have not observed any of them.

According to our investigations, the nerve fibres connected with the chromaffin cells are all nonmyelinated. These grow towards the chromaffin cells wrapped in SCHWANN's cells, here in the same way as everywhere. Both the cell membrane and the wall of the mesaxon duct are thin but extremely sharp. In the cross-section of the axon, the cross-sections of neurofilaments and the synaptic vesicles appear clearly. The latter ones are light but there are to be seen among them, in an extremely low number, some forms of dense core type, as well.

Synapses

The axons leaving SCHWANN's cells are in a synaptic connection with the chromaffin cells. We have found two forms of synapses. One of them takes place on the surface of cell, is roundish and of major size, the other lies near the nuclear membrane. On the first one there are to be seen synaptic membranes having synaptic thickenings extending over smaller parts, too. The mitochondria are long-shaped discs, in a very low number. The synaptic vesicles are roundish, distributed uniformly in the central part of the axon-end; in the part of the periphery, however, where the synaptic membranes are thickened, the cluster marking the direction of the process of impulse can be seen (Fig. 9). The second form of synapses is long-shaped, in the axon-end there are several mitochondria, they are elongated and belong to the crista type. The synaptic vesicles are small but also larger forms are to be seen, mainly in the periphery. The small vesicles form sporadically groups. These groups occur both in the central and in the peripheral parts of the axon. The synaptic membranes are thickened on some places only. The single parts thickened are short and thick and the synaptic membranes are grown together in them (Fig. 7).

The extremely intimate form of the connection between the chromaffin cells and the nervous system is justifying the sudden changes in the hormone production, their course depending strongly upon the nervous system and the environmental influences and their intensity.

In the interrenal substance we could find neither any nerve fibre nor any synapsis. By this finding there is justified our old light microscopic establishment according to which the interrenal section of the adrenal gland, apart from a few nerve fibres leading perpendicularly through it that at *Mammalia* manifest themselves in some cases, seems to be thoroughly nerveless, while the adrenal substance enjoys a rich and deep seated innervation.

Erythrocytes

Both the cytoplasm and the nucleus of erythrocytes are homogeneous, respectively full of dense and small granules. Among the components of cytoplasm we have to deal with the so-called marginal band (Border stria, Randreifen), if only because of its interesting career. The formation was seen first probably by RANVIER (1875) who described an uncommon thick membrane from the periphery of the stained erythrocytes of *Amphibia* that covered the whole surface of body. Independently of him, DEHLER (1875) published dark rings from the erythrocytes of duck embryos after staining with iron hematoxin, the thickness of which changed 0.3 and 1.5 μ . DEHLER regarded these forma-

tions as independent of the cell membrane and as a thickening of the surface part of cytoplasm. A similar structure was found by HEIDENHAIN (1896) among the erythrocytes of *Amphibia*, NICHOLAS (1892) among those of *Reptilia*. All these authors have found essentially a homogeneous band that surrounds the cytoplasm of erythrocyte taking place immediately under the cell membrane. MEVES (1904) demonstrated on the basis of a supravital staining that in the erythrocytes of *Amphibia* the marginal band was composed of fibrils. MEVES thought this structure elastic supposing that it had an important role in

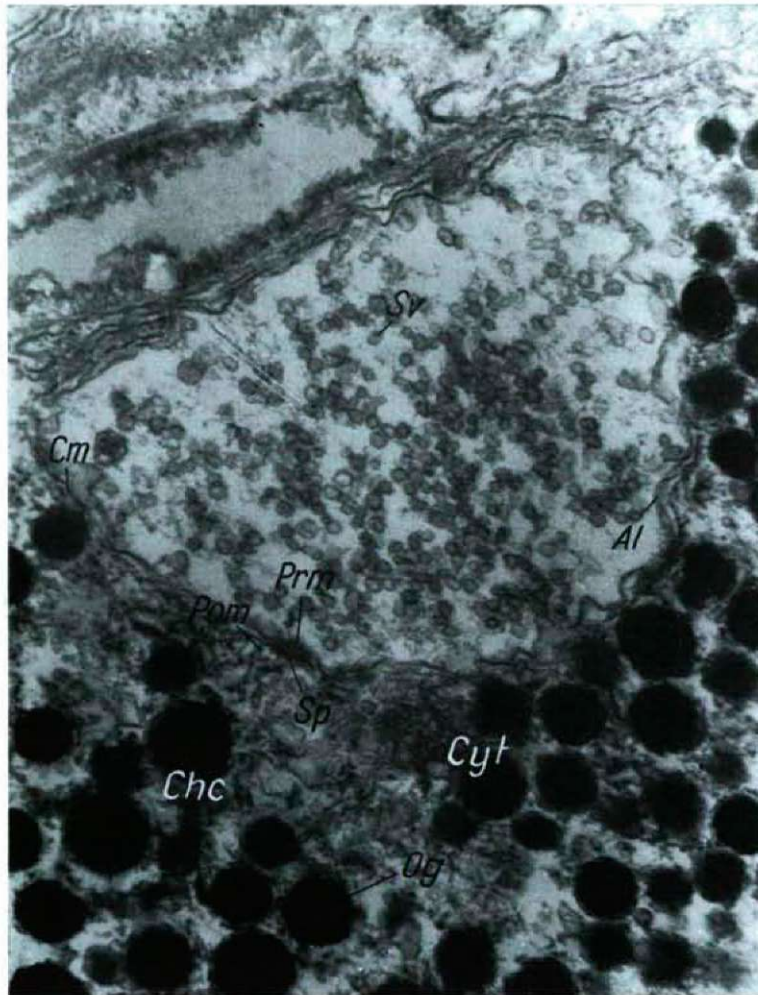


Fig. 9. *Bufo viridis* LAUR. Synapsis on the chromaffin cell of the adrenal gland. Chc./chromaffin cell, Cyt./cytoplasm, Og./osmiophilic body, Sv./synaptic vesicle, Prm./pre-synaptic membrane, Pom./post-synaptic membrane, Sp./synaptic space, Al./axolemma, Cm./cell membrane. Magnified: x 75.000

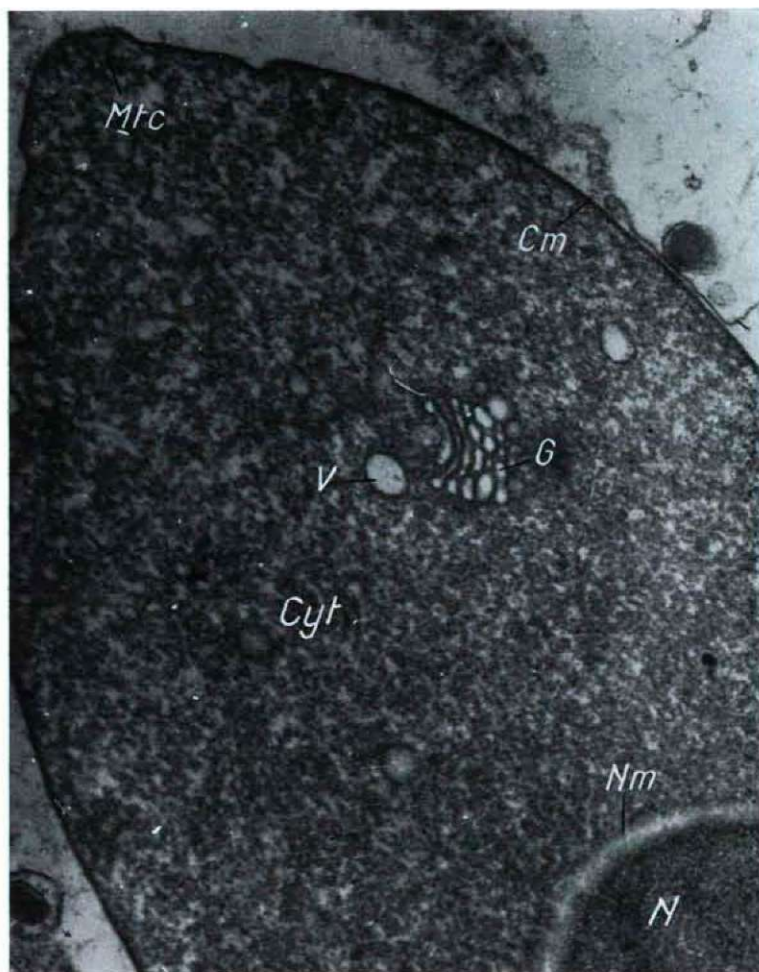


Fig. 10. *Bufo viridis* LAUR. Erythrocyte. Cyt./cytoplasm, N./nucleus, Nm./nuclear membrane, Cm./cell membrane, G./Golgi apparatus, Mtc./marginal tubules in cross-section. Magnified: $\times 40.000$

preserving the characteristic flattened form of cells. WEIDENREICH (1905) qualified the striation published by MEVES rugosity, resp. corrugation, produced on the surface of cell as a result of stain solutions. Therefore he described the marginal band simply as an artificial production. WEIDENREICH's opinion was generally accepted by the scientists and the marginal band has disappeared from the hematological literature since 1910. The interest in this mysterious structure was only excited again as TROTTER (1956) investigating the form-alterations of erythrocytes of the yellow-and black-spotted land-salamander under experimental conditions, had come to the conclusion that the cells must have some special structure limiting their flexibility and taking part in the

preservation of their shape, resp. in restituting it. He considered, therefore, MEVES's establishments as correct and restored the marginal band in the literature. FAWCETT (1959) obtained several erythrocyte sections during the electron microscopic investigation of the miraculous rete (rete mirabile) of the swim-bladder of fish that are favourable to the theses of HEIDENHAIN and MEVES because under the cell membrane there is actually an elastic mechanism that may have a considerable part in preserving the discoid or ellipsoid form of erythrocytes. FAWCETT and WITEBSKY (1964) found two groups consisting of small nodes under electron microscope with weak magnification at the two poles of erythrocytes of toad-fish (*Optanus tau*) at the place where the cross-section of the marginal band is to be looked for. They ascertained in pictures more magnified that the nodes forming the single groups are, as a matter of fact, nothing else but empty fibrils or the cross-sections of tubuli running under the cell membrane, the diameter of each of which is 200 Å. They found in each group 24 to 28 circular cross-section. The number of the smallest was 10, that of the largest 30.

We, too, have found the cross-sections of FAWCETT and WITEBSKY's (1964) tubules in the erythrocytes of the green toad (Fig. 10). These take place in the pointed pole of the haemocyte where close to the cell membrane they form groups. From the elongatedly roundish tubule cross-sections of proportionately thick wall the conclusion can be drawn that there are real tubule groups here, running around in the polar part of the cell. It is also possible, anyway, that they end blind. And it is, of course, also not excluded that they only run through the cells, having two openings. We have found the cross-sections of fifteen tubules in one group. We don't know what purpose they serve. But combining our investigations with those of FAWCETT's, we believe not to be far from truth if we say that in the erythrocytes of the submammalian *Vertebrata* (*Pisces*, *Amphibia*, *Reptilia*, *Aves*) a tubule system has taken place under the cell membrane making possible a high degree of elasticity and playing an important part in restoring the changes in place induced by external effects.

In the cytoplasm there are sometimes to be seen roundish vesicles including some particles bordered with sharp walls. Besides them there occur also erythrocytes, if only but quite rarely, in which GOLGI's complex appears in its peculiar sharpness and characteristic (Fig. 10).

The cytoplasm is separated from the homogeneous and electron dense nucleus by a wide light space of changing thickness. There isn't any solid nuclear membrane.

Summary

As a result of the investigations carried out with electron microscope on the adrenal gland of the green toad (*Bufo viridis* LAUR.) there were established the followings:

1. The cellular elements of adrenal gland are the lipid cells, the chromaffin cells and the acidophilic cells.
2. The lipid cells are roundish bodies of loose structure, their cytoplasm is full of lipid bodies bordered with wavy walls. They are characterized by large mitochondria of tubule type small ovoid vesicles and by free ribosomes being sporadically present en masse.

3. The forms being transformed are not rare among the mitochondria. In these the tubules that are generally scattered or form in some cases groups immediately in the vicinity of the wall become arranged sheaf-like, then they fall to smaller or larger pieces, melting into an electron dense mass without any structure.

4. The chromaffin cells are long-shaped bodies, sharpened at their both ends. In their cytoplasm there are a large number of osmiophilic bodies. Some of these are homogeneous, others contain small granules. The homogeneous ones belonging to the dense core type. The mitochondria are much smaller than in the lipid cells and also their number remains strongly under that of the latter ones.

5. The acidophilic cells, resp. the summer cells are elliptical bodies that are but rarely to find, containing many mitochondria, few osmiophilic bodies, these, however, are much larger than those in the chromaffin cells.

6. The naked axons are connected with the chromaffin cells by means of two synapsis forms. One of them takes place on the surface of the cell, is full of clear synaptic vesicles but missing mitochondria. The membrane thickenings are limited to a single short sector and the synaptic space is obvious. The other synaptic form takes place in the next vicinity of the cell nucleus, in the axon-end there are many mitochondria and synaptic vesicles, the synaptic membranes are melted and thickenings are only on some places.

7. In the pole of erythrocytes, under the cell membrane a circular marginal tubule system takes place, playing the part of a cell framework and providing the cell elasticity. The tubules of Golgi apparatus are parallel, their course is obvious and their dilatations are large.

References

- ÁBRAHÁM, A. (1963): The nerve supply of the adrenal gland in birds. — *General and Comp. Endocrinol.* 3, p. 680.
- ÁBRAHÁM, A. (1966): Nerve supply of the adrenal gland. — *Acad. Bulgare des Sciences V. Symp. histol. internat. Sophia* 289—304.
- ÁBRAHÁM, A. (1969): Electron microscopic observations on the medial neurosecretory cells in the brain of the water beetle (*Dytiscus marginalis*). — *Z. mikr.-anat. Forsch.* 80, 469—484.
- BACHMANN, R., SCHARRER, E. und SCHARRER, B. (1954): Die Nebenniere. Neurosecretion. In: MÖLLENDORFF: *Hb. der mikr. Anat. des Menschen* 6/. — Springer, Berlin, Göttingen, Heidelberg.
- BLOCH, B., THOMSEN, E. and THOMSEN, M. (1966): The neurosecretory system of the adult *Calliphora erythrocephala* III. Electron microscopy of the medial neurosecretory cells of the brain and some adjacent cells. — *Z. Zellforsch.* 70, 185—208.
- BONNAMOUR, A., OPLICARD, A. (1903): Note histologique sur la capsule surrénale de la grenouille. — *C. r. Assoc. Anat.* 102—103.
- BURGOS, H. M. (1959): Histochemistry and electron microscopy of the three cell types in the adrenal gland of the frog. — *Anat. Record* 133, 163—186.
- CIACCIO, C. (1903): Ricerche sui processi di secrezione cellulare nelle capsule surrenali dei vertebrati. — *Anat. Anz.* 23, 404—424.
- FAWCETT, W. DON. (1959): Electron microscopic observations on the marginal band of nucleated erythrocytes. — *Anat. Rec.* 133, 379.
- FAWCETT, W. DON. and WITEBSKY, F. (1964): Observations on the ultrastructure of nucleated erythrocytes and thrombocytes, with particular reference to the structural basis of their discoidal shape. — *Z. Zellforsch.* 62, 785—806.
- GRYNFELT, E. (1904): Notes histologique sur la capsule surrénale de la grenouille (*Rana esculenta*). — *C. R. Soc. Biol. Paris* 120, 425—465.

- KRAUSE, R. (1923): Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. *Amphibien*. — Walter de Gruyter et Co. Berlin und Leipzig.
- KUCNEROWICZ, H. (1935): Sur les „cellules d'été" dans la glande surrénale de la grenouille (*Rana esculenta*). — C. R. Soc. Biol. Paris 120, 486—491.
- LEVER, J. D. (1955): Electron microscopic observations on the adrenal cortex. — Am. J. Anat. 97, 409—429.
- PEHLEMANN, F. W. (1968): Die amitotische Zellteilung. Eine elektronmikroskopische Untersuchung an Interrenal-Zellen von *Rana temporaria*. — Z. Zellforsch. 84, 516—548.
- RADU, V. (1931): Etude cytologique de la glande surrénale des amphibiens anoures. — Bull. d'Histol. Appl. 8, 249—264.
- SCHARRER, E., BROWN, S. (1961): Neurosecretion XII. The formation of the neurosecretory granules in the earthworm (*Lumbricus terrestris*). — Z. Zellforsch. 54, 761—796.
- SINGER, E., and ZWENNER, R. L. (1934): Microscopic observations of structural changes in the adrenal gland of the living frog under experimental conditions. — Anat. Rec. 183—187.
- STILLING, E. (1898): Zur Anatomie der Nebennieren. — Arch. mikr. Anat. 52, 176—195.

Address of the author:
Prof. Dr. A. ÁBRAHÁM
Department of Zoology,
A. J. University, Szeged,
Hungary